Synthesis and biological activity of new C-6 and C-7 substituted vinyloxyimino-penicillins and -cephalosporins

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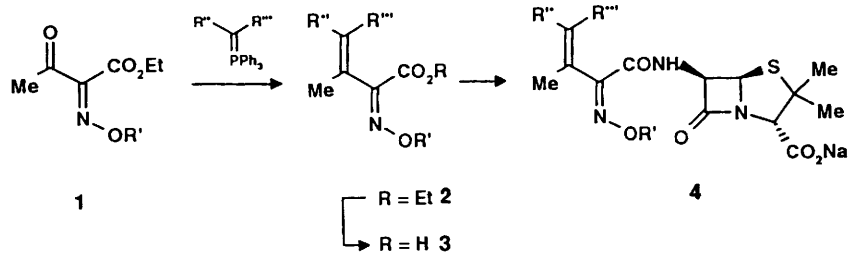
The Wittig reaction has been successfully utilized in the preparation of a number of substituted α-vinyloxyiminoacetic acids, the subsequent coupling of which to the 6-APA and 7-ACA nuclei has provided a range of potent β-lactam antibiotics. Development of other procedures for olefin synthesis has broadened the scope, with the preparation of a range of alkenyl-, cycloalkenyl- and arylvinyl-oxyiminoacetamidopenicillins and cephalosporins.

A recent report from these laboratories[1] described a series of (*Z*)-2-alkoxyimino-2-(2-aminothiazol-4-yl)acetamidopenicillins which showed high stability to β-lactamases and potent antibacterial activity against Gram-positive and certain Gram-negative organisms. Concurrently we were investigating the replacement of the aminothiazolyl group with a substituted double bond.

We report here the synthesis of some novel 2-oxyiminovinylacetamido-penicillins and -cephalosporins and, in particular, the substituted 2-oxyiminovinylacetic acids used in their preparation.

Initial studies employed the readily available ethyl (*Z*)-2-alkoxyiminoacetoacetate **1** used in the preparation of (*Z*)-2-alkoxyimino-2-(2-aminothiazol-4-yl)acetic acids. Thus, Wittig reaction of ethyl (*Z*)-2-methoxyiminoacetoacetate[2] **1** (R′ = Me) with a range of triphenylphosphoranes provided the corresponding alkenes **2**. After hydrolysis of the ester function of **2** and conversion of the derived acids **3** into acid chlorides, acylation of 6-aminopenicillanic acid (6-APA), with the latter, gave the penicillins (**4a–c**) (Scheme 1). In the same way a variety of alkoxyimino examples **4d–g** were prepared (Table 1). For examples **2a–e** the phosphorane precursors were generated *in situ* from the corresponding phosphonium salts with butyllithium in tetrahydrofuran (THF) at 0 or −10 °C. The dichloro analogues **2f, g** were prepared by reaction of the keto ester **1** (R′ = Me or Bu^t) with triphenylphosphine in refluxing carbon tetrachloride, although the yields were generally poor.

Encouraged by the level of antibacterial activity shown by these penicillins, we sought to extend the range of substitution on the double bond. The 2-(2-aminothiazol-4-yl)acetamido group is well established as providing advantageous properties to penicillins and cephalosporins.[3] Therefore, as vinylogues, the oximes **7** (Table 2) were prepared by the Wittig condensation of **1** with the appropriate phosphorane generated from the salt **6**, formed from the chloride[4] **5**. The phosphoranes were generated with potassium *tert*-butoxide in THF at room temperature (**7a**) or *in situ* with potassium carbonate in *N*,*N*-dimethylformamide (DMF), either at room temperature or 80 °C (**7b, c**). Generally the yields in these cases were poor, but hydrolysis to the acids **8**, followed by coupling to 6-APA gave the penicillins **9a–c**. For



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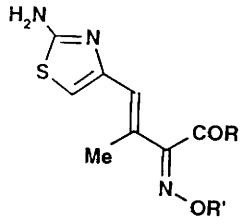
Scheme 1

Table 1

	R′	R″	R‴	Yield (%) Ester **2**	Acid **3**	Penam **4**
a	Me	H	H	33	96	43
b	Me	Me(H)[a]	H(Me)[a]	46	89	63
c	Me	Ph	H	31	77	46
d	$CH_2CH_2CH_2CH_2$	H	H	39	70	35
e	c-C_6H_{11}	H	H	54	30 (66)[b]	74
f	Me	Cl	Cl	28	96	45
g	Bu^t	Cl	Cl	13	98	54

[a] A 1:1 mixture of *E* and *Z* isomers, inseparable at all stages. [b] Yield in parentheses *via* general procedure [F].

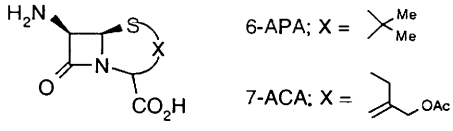
Table 2



	R′	Yield (%) 7 R = OEt	8 R = OH	9 R = APA/ACA
a	Me	41 (28)[a]	89	18
b	C_6H_{11}	26	43	57
c	Bu^t	14	60	61
d	Me			34

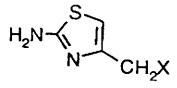
[a] Yield in parentheses, general procedure (B).

comparison the methoxyimino derivative **8a** was also coupled to 7-aminocephalosporanic acid (7-ACA) to give the cephalosporin **9d**.

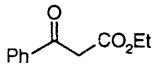


6-APA; X =

7-ACA; X =



5 X = Cl
6 X = $PPh_3^+Cl^-$



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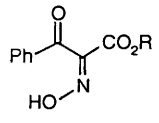
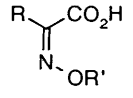
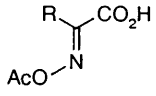


11 R = Et
12 R = H

The successful synthesis of alkenes of type **2c** and **7** led us to consider the preparation of other aromatic analogues. The oximation of ethyl benzoylacetate **10** with sodium nitrite in acetic acid was of particular interest, since the reaction gave exclusively the *E*-oxime **11**, in contrast to the *Z*-oxime usually formed with acylacetates. The stereochemistry was confirmed by employing the observation that *Z*-acetoxyimino acids of the type **13** are hydrolysed with sodium hydroxide to give the hydroxylamines **14**, whereas the *E*-isomers **15** undergo *trans* decarboxylative elimination to give nitriles.[5] When **12** was



13 R′ = Ac
14 R′ = H



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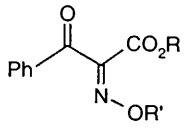
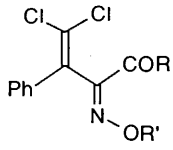
treated with acetyl chloride in the presence of dimethylaminopyridine, the only product isolated was benzoyl nitrile.

Fortunately, however, the oxime **11** could be partially isomerised with 5% palladium-on-alumina in refluxing benzene resulting in a 2:1, *E*:*Z* mixture. The required *Z* isomer **16** was isolated from the mixture by silica gel chromatography. Methylation with iodomethane and potassium carbonate gave **17**, from which the dichlorovinyl compound **18** was obtained with triphenylphosphine in refluxing carbon tetrachloride. Subsequent hydrolysis to **19** and coupling to 6-APA afforded the 2,2-dichloro-1-phenyl substituted penicillin **20**.



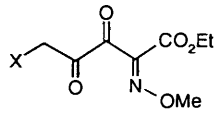
16 R = Et R′ = H
17 R = Et R′ = Me



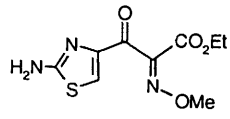
18 R = OEt R′ = Me
19 R = OH R′ = Me
20 R = APA R′ = Me

The antibacterial properties of this 2,2-dichloro-1-phenyl analogue **20** prompted us to consider possible routes to the corresponding derivative in which the 2-aminothiazol-4-yl group replaced the phenyl ring.

Initially, the α-diketone **21** was targeted as an appropriate precursor to the aminothiazole derivative **22**. Ethyl 3-oxopentanoate was converted into the *Z*-methoxyimino compound **23** under the usual conditions; successive bromination to **24** followed by treatment with silver nitrate in acetonitrile gave **25**. Without purification this was converted into the desired α-diketone **21** by the elimination of nitrite ion with sodium acetate in dimethyl sulfoxide (DMSO).[6] Unfortunately, the α-diketone **21** proved to be unstable and several methods of bromination, followed by reaction with thiourea failed. The bromide **26**, was eventually obtained by the reaction of *N*-bromosuccinimide (NBS) with the trimethylsilyl enol ether of **21**. Condensation of the unpurified bromide with thiourea gave **22**, albeit as a 2:1



21 X = H
26 X = Br



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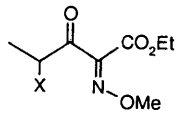
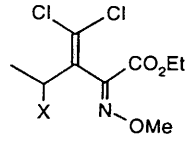
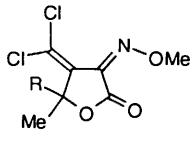


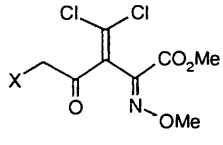
23 X = H
24 X = Br
25 X = ONO_2



27 X = H
28 X = Br
29 X = $OCOCF_3$



30 R = H
31 R = Br



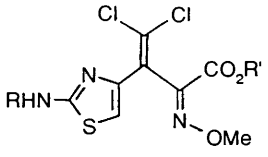
32 X = H
33 X = Br

mixture of oxime isomers, separation of which by crystallization from ether gave a low yield of a single pure isomer of **22** of undefined stereochemistry. The last-mentioned feature together with the poor yield of **22** and its inability to react with triphenylphosphine in carbon tetrachloride under a variety of conditions, led us to investigate alternative routes.

We turned our attention to the possibility of forming the

dichlorovinyl function prior to cyclization to the aminothiazole derivative. Hence the oxime **23**, was converted into **27** and, following allylic bromination with NBS, **28** was obtained. Treatment of this with silver trifluoroacetate in acetonitrile afforded **29** but, interestingly, hydrolysis with sodium hydrogen carbonate gave only the lactone **30**. Ring opening with sodium hyroxide was followed by spontaneous ring closure when the solution dropped below pH 7. Fortuitously, the lactone **30** also underwent bromination with NBS to furnish **31**. In this case, ring opening with aqueous sodium hydrogen carbonate resulted in collapse to the ketone, and, following *in situ* esterification of the resultant acid, gave the desired ester **32**. This upon bromination gave **33**, which reacted with thiourea to afford the side chain ester **34**. Protection of the 2-amino group of the heterocyclic ring of this was found to be necessary for the subsequent coupling reaction of the derived acid to 6-APA.

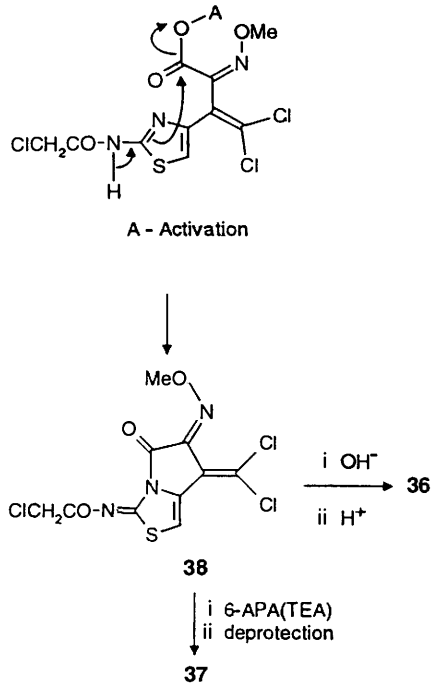
Consequently, compound **35**, prepared by reaction of **34** with chloroacetyl chloride in the presence of triethylamine, was hydrolysed to the acid **36** with sodium hydroxide in high yield. The Vilsmeier coupling of this acid with the triethylamine salt of 6β-aminopenicillanic acid [6-APA(TEA)] is worthy of a special mention. Activation of the acid with oxalyl chloride and DMF in dichloromethane at −10 °C resulted in the immediate precipitation of an intense yellow solid. Since after 30 min with 6-APA(TEA) at room temperature there appeared to be no



34 R = H R' = Me
35 R = $ClCH_2CO$- R' = Me
36 R = $ClCH_2CO$- R' = H
37 R = H R' = APAONa

further reaction, the solid was filtered off and dried. Some penicillin product was isolated from the filtrate, and deprotection with sodium *N*-methyldithiocarbamate [1] followed by purification gave the penicillin **37** in 17% yield. The yellow solid was insoluble in most organic solvents, but readily gave a colourless solution in dilute aqueous sodium hydroxide. Acidification of this gave a white solid which was consistent with the authentic acid **36**. The mass spectrum of the yellow solid gave M^+ 353 and an accurate mass measurement of 353.9188, consistent with the empirical formula $C_{10}H_6Cl_3N_3O_3S$. Rationalization of this observation suggests that activation of the acid **36** is followed by spontaneous cyclization to the bicyclic system **38**. It is interesting that this cyclization mimics the lactone formation observed during hydrolysis of **29** to **30**. In both cases it is believed that the driving force is the planar, sp^2 nature of the three adjacent carbon atoms. Since the ring amide can be readily hydrolysed to the acid **36**, then other nucleophiles may also result in ring opening. Indeed, a suspension of **38** in dichloromethane at room temperature with 6-APA(TEA), eventually resulted in a colourless homogeneous solution. TLC analysis showed complete formation of the penicillin product. Clearly had the initial reaction been allowed to progress, complete reaction would have been observed.

Having successfully completed the synthesis of a number of substituted vinyl oximes including some novel aromatic and heterocyclic analogues, we decided to extend the investigation by synthesizing a variety of non-aromatic cyclic alkenyl oximes. For these we utilized the versatile methyl dimethoxyacetate,[7]



A - Activation

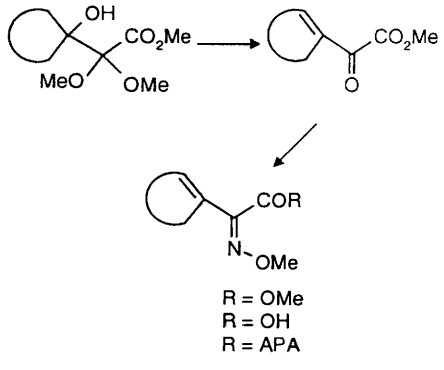
38

37

Table 3

	Ketone	Yield (%) 39	40	41	42	43
a	$CH_2(CH_2)_4CO$	67	88	86	92	78
b	$CH_2(CH_2)_3CO$	52	24	67	99	55
c	$CH_2CH_2O(CH_2)_2CO$	70	44	75	100	41

which has been used as a synthon for various alkenyl glyoxylate esters.[8] The reagent underwent lithiation with either lithium diisopropylamide or butyllithium and reacted with cyclic ketones to give the corresponding hydroxy intermediates **39**. Dehydration with thionyl chloride and pyridine followed by *in situ* acid hydrolysis gave the required cycloalkenyl glyoxylates **40**. Formation of the oximes **41** with methoxylamine hydrochloride, and coupling to 6-APA *via* **42** afforded the requisite penicillins **43** (Scheme 2). Three examples, including one heterocyclic derivative are shown in Table 3.



R = OMe
R = OH
R = APA

Scheme 2

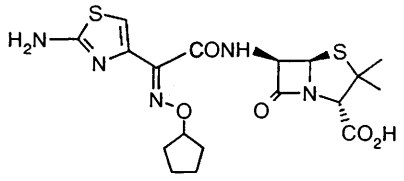
Biological data

Table 4 shows some typical *in vitro* minimum inhibitory concentrations (MIC) values (μg cm^{-3})* for a range of organisms. The penicillin, BRL 44154 [1] (see structure **44**) has

Table 4

Organism	4f	9a	37	43a	BRL 44154 44[1]
Haemophilus influenzae Q1	0.5	0.25	2	0.5	
H. influenzae NEMC1 +	8	4	4	16	
Escherichia coli ESS	1	0.25	2	0.25	
Moraxella catarrhalis Ravasio +	1	8	32	32	0.5
Staphylococcus aureus Oxford	0.25	0.5	4	0.5	0.25
S. aureus Russell +	2	1	4	2	0.5
S. aureus MB9 +	> 128	2	8	4	
S. epidermidis PHLN20	4	0.5	8	0.5	0.25
Streptococcus pyogenes CN10	< 0.06	< 0.06	0.25	0.12	
S. pneumoniae 1761	< 0.06	< 0.06	0.5	0.12	⩽0.03
S. pneumoniae PU7 ‡	4	4	64	16	
S. agalactiae 2798	0.25	0.25	4	0.5	

* Serial dilution in blood agar base (Oxoid) containing 5% lysed horse blood. Inoculated with 0.001 cm^3 of an overnight broth culture diluted as appropriate. + β-Lactamase mediated resistance. ‡ Target site-mediated resistance.



44 BRL 44154

been included with a selected number of organisms for comparison.

Summary

We have demonstrated that valuable intermediates in the synthesis of potent, third-generation cephalosporin antibiotics can also be employed in the synthesis of new vinyloxyimino β-lactams which maintain a good level of antibacterial activity. Furthermore, development of this concept has allowed us to produce a wide range of penicillin and cephalosporin antibiotics.

Experimental

Mps were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded on Perkin-Elmer 197 or 983 spectrophotometers. Proton NMR spectra were recorded on Perkin-Elmer R12 (60 MHz), R32 (90 MHz) or Bruker AM 250 (250 MHz) spectrometers. Chemical shifts are quoted in ppm relative to tetramethylsilane as an internal reference for solutions in $CDCl_3$ or $(CD_3)_2SO$ and external HOD set at δ 4.80 for solutions in D_2O. J Values are in Hz. Mass spectra, electron impact (EI), chemical ionization (CI) using ammonia and fast-atom bombardment (FAB) using thioglycerol, were obtained on VG 7070F or VG ZAB 1F mass spectrometers. Microanalytical data were determined on a Carlo Erba 1106 elemental analyser. Removal of solvents under reduced pressure was performed using a rotary evaporator. Chromatography was performed using Merck silica gel 60 (9385) and (7729). Sodium salts of β-lactams were purified using Mitsubishi Diaion HP20SS using THF/water mixtures. Aqueous solutions of the β-lactams were freeze-dried, and shown to be homogeneous by reverse phase HPLC.

General procedures

(A) Wittig condensations with butyllithium. A suspension of the triphenylphosphonium salt (1 equiv.) in dry tetrahydrofuran (0.5 mmol cm^{-3}) was cooled to −10 °C and treated with butyllithium (1.6 mol dm^{-3} solution in hexane; 1 equiv.). The reaction mixture was warmed to room temperature for 20–30 min until a homogeneous solution was obtained after which it was cooled again to −10 °C. After addition of the ketone (1 equiv.) to it, the reaction mixture was maintained at room temperature until TLC analysis showed completion of the reaction. The reaction was then quenched by the addition of saturated aqueous ammonium chloride to the mixture after which the product was extracted with ethyl acetate. The extract was dried ($MgSO_4$) and evaporated under reduced pressure to provide a residue which was purified by silica gel chromatography to afford the desired products.

(B) Wittig condensations with potassium *tert*-butoxide. A suspension of the triphenylphosphonium salt (1 equiv.) in dry THF (0.25 mmol cm^{-3}) was treated with potassium *tert*-butoxide (1 equiv.) and the mixture heated to 60 °C until a homogeneous solution was obtained. After cooling of the mixture to room temperature, the ketone (1 equiv.) was added to it and the reaction monitored until it was complete. After removal of the excess of THF under reduced pressure, the product was isolated as described in general procedure (A).

(C) Wittig condensations with potassium carbonate in DMF. A suspension of the phosphonium salt (1.2 equiv.) in DMF (0.5–0.8 mmol cm^{-3}) was treated with the ketone and potassium carbonate (1.2 equiv.) and maintained at room temperature for 2–3 days. If little or no reaction was observed by TLC analysis the reaction mixture was heated to 80 °C until completion of the reaction. The solution was then diluted with ethyl acetate, washed with water (× 3) and brine, dried ($MgSO_4$), filtered and evaporated under reduced pressure. The product was purified by silica gel chromatography.

(D) Wittig condensations with triphenylphosphine in carbon tetrachloride. The ketone and triphenylphosphine (2 equiv.) in carbon tetrachloride (0.2–0.5 mmol cm^{-3}) was heated under reflux for 5–6 h, after which the reaction mixture was cooled and the supernatant liquid evaporated. The residue was extracted with hexane and the extract evaporated under reduced pressure to provide a residue. This was purified by silica gel chromatography to afford the product.

A modification[9] of this procedure involved the use of acetonitrile as the solvent, with carbon tetrachloride (2 equiv.) and triphenylphosphine (2 equiv.). The reaction mixture was maintained at room temperature. Complete removal of the solvent under reduced pressure allowed the residue to be purified as before.

(E) Hydrolysis of esters with sodium hydroxide. The ester dissolved in ethanol (0.5–1.5 mmol cm^{-3}) was treated at room temperature with an excess (3 equiv.) of 1 mol dm^{-3} aqueous sodium hydroxide. After completion of the reaction as adjudged by TLC analysis, the solution was concentrated by removal

of ethanol, diluted with water, acidified with dilute hydrochloric acid and extracted with ethyl acetate. The extract was dried and concentrated by removal of solvent to give the crude acids which were sufficiently pure for direct use without further purification.

(F) Hydrolysis of esters with potassium carbonate. The ester in an excess of 10% aqueous methanol (0.1–0.5 mmol cm^{-3}) was treated with potassium carbonate (2 equiv.) and the mixture heated under reflux until the reaction was complete. The solution was then cooled, diluted with water and adjusted to pH 2 with dilute hydrochloric acid. The acid was isolated as described in general procedure (E).

(G) Couplings with 6-APA. (i) Via *The acid chloride.*—The acid in methanol (0.5–1 mmol cm^{-3}) was converted into its sodium salt with sodium methoxide (1 equiv.) in methanol (0.5–1 mmol cm^{-3}) after which the solvent removed under reduced pressure. A suspension of this salt in dichloromethane (1 mmol cm^{-3}) was treated with oxalyl chloride (1.1 equiv.) with a trace of DMF. The excess of oxalyl chloride was removed under reduced pressure and the crude acid chloride redissolved in fresh dichloromethane (1 mmol cm^{-3}). This solution at 0 °C was treated with a solution of triethylammonium 6β-aminopenicillanate [6-APA(TEA); 1.2 equiv.] and triethylamine (1.2 equiv.) in dichloromethane (0.2 mmol cm^{-3}). After warming to room temperature for 30 min, the mixture was concentrated by removal of solvent and this was replaced with ethyl acetate–water. After the mixture had been adjusted to pH 2 with dilute hydrochloric acid, the organic phase was separated, dried and concentrated to afford the crude penam. This, in ether was converted into its sodium salt with sodium 2-ethylhexanoate (1 equiv.) in isobutyl methyl ketone (IBMK). The isolated product was purified by HP20SS column chromatography. The penicillin sodium salts were isolated from the aqueous solutions by freeze-drying.

(ii) Via *Vilsmeier coupling.*—A solution of DMF (1 equiv.) in dichloromethane (0.25 mmol cm^{-3}), cooled to −10 °C was treated with oxalyl chloride (1 equiv.) for 10 min after which a solution of the acid (1 equiv.) in dichloromethane (1 mmol cm^{-3}) was added to it. This was followed after a further 10 min by 6-APA(TEA) (1.2 equiv.) and triethylamine (1.2 equiv.) in dichloromethane (1 mmol cm^{-3}) after which the solution was allowed to warm to room temperature. After isolation of the crude acid, as described in (G)(i) it was dissolved in saturated aqueous sodium hydrogen carbonate and subjected to HP20SS chromatography as described.

(iii) Via *Mixed anhydride coupling.*—A solution of the acid (1 equiv.) in dry DMF (0.2 mmol cm^{-3}), under argon was cooled to −50 °C and treated with *N*,*N*-diisopropylethylamine (1.1 equiv.) followed by methanesulfonyl chloride (1.1 equiv.). After 40 min, a solution of 6-APA(TEA) (1.2 equiv.) and triethylamine (1.2 equiv.) in dichloromethane 0.2 mmol cm^{-3} was added to the reaction mixture which was then allowed to warm to room temperature. After this the mixture was concentrated and partitioned between ethyl acetate–water. The aqueous phase was separated and further extracted with butanol. The combined organic phases were dried ($MgSO_4$) to give, after work-up, the crude penicillin which was purified as described in general procedure (G)(ii).

Ethyl (*Z*)-2-methoxyimino-3-methylbut-3-enoate 2a. General procedure (A). Ethyl (*Z*)-2-methoxyimino-3-oxobutanoate[2] **1a** (1.73 g), gave the *title compound* as a colourless oil (0.56 g, 33%) (Found: M^+, 171.0898. $C_8H_{13}NO_3$ requires *M*, 171.0895); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1730, 1620w, 1575w and 1450; $\delta_H(CDCl_3)$ 1.35 (3 H, t, *J* 7.3, CH_2CH_3), 1.97 (3 H, br s, =C–CH_3), 3.95 (3 H, s, OCH_3), 4.38 (2 H, q, *J* 7.3, CH_2CH_3), 5.18 (1 H, br s, =CH) and 5.44 (1 H, br s, =CH).

(*Z*)-2-Methoxyimino-3-methylbut-3-enoic acid 3a. General procedure (E). Compound **2a** (0.54 g) was hydrolysed to give the *title compound* as a colourless oil which partially crystallized (0.43 g, 96%) (Found: M^+, 143.0582. $C_6H_9NO_3$ requires *M*, 143.0582); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3650w, 3450w, 3300–2600v, br, 1755 and 1720; $\delta_H(CDCl_3)$ 1.99 (3 H, br s, =CCH_3), 4.03 (3 H, s, OCH_3), 5.36 (1 H, br s, =CH), 5.50 (1 H, br s, =CH) and 11.02 (1 H, br s, CO_2H).

Sodium 6β-[(*Z*)-2-methoxyimino-3-methylbut-3-enamido]-penicillanate 4a. As outlined in general procedure (G)(i), the acid **3a** (0.39 g) was coupled to triethylammonium 6β-aminopenicillanate [6-APA(TEA)]. After purification, the *title compound* was obtained as an amorphous, freeze-dried solid (0.41 g, 43%); ν_{max}(KBr)/cm^{-1} 1765, 1622, 1605, 1538, 1456 and 1400; $\delta_H(D_2O)$ 1.51 (3 H, s, 2-CH_3), 1.62 (3 H, s, 2-CH_3), 1.93 (3 H, s, =CCH_3), 3.92 (3 H, s, OCH_3), 4.24 (1 H, s, 3-H), 5.37 (1 H, s, =CH), 5.57 (1 H, d, *J* 1.3, =CH) and 5.61 (2 H, s, 5- and 6-H); *m*/*z* (positive ion FAB, thioglycerol) 364 (MH^+), 386 (MNa^+) and 749 (2M + Na^+).

(*Z*)-2-Methoxyimino-3-methylpent-3-enoic acid 3b. Using general procedure (A), **1a** (1.73 g) was converted into the ester **2b**, a colourless oil (0.86 g, 46%). Then using general procedure (E), the ester **2b** was hydrolysed to the *title compound*, a colourless oil (0.49 g, 89%) (Found: M^+, 157.0729, $C_7H_{11}NO_3$ requires *M*, 157.0739); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3630w, 3450w, 3300–2700br, 1750 and 1720; $\delta_H(CDCl_3)$ 1.72–2.00 (6 H, m, 2 × CH_3, *E* and *Z* isomers), 3.98 and 4.00 (3 H, 2s, OCH_3, *E* and *Z* isomers), 5.71–6.07 (1 H, m, =CH–, *E* and *Z* isomers) and 10.72 (1 H, s, CO_2H, *E* and *Z* isomers).

Sodium 6β-[(*Z*)-2-methoxyimino-3-methylpent-3-enamido]-penicillanate 4b. Following general procedure (G)(i), the acid **3b** (0.49 g) was coupled to 6-APA(TEA). Isolation and purification gave the *title compound*, as an amorphous solid, obtained as a 1:1 mixture of *E* and *Z* isomers (0.67 g, 63%); ν_{max}(KBr)/cm^{-1} 1766, 1662, 1603, 1515, 1399 and 1319; $\delta_H(D_2O)$ 1.51 (3 H, s, 2-CH_3, *E* and *Z* isomers), 1.62 and 1.63 (3 H, 2s, 2-CH_3, *E* and *Z* isomers), 1.79–1.85 (6 H, m, 2 × CH_3, *E* and *Z* isomers), 3.88 and 3.91 (3 H, 2s, OCH_3, *E* and *Z* isomers), 4.23 and 4.24 (1 H, 2s, 3-H, *E* and *Z* isomers), 5.58 and 5.60 (2 H, ABq, *J* 4.7, 5- and 6-H, *E* and *Z* isomers) and 5.94–5.99 (1 H, m, =CH, *E* and *Z* isomers); *m*/*z* (positive ion FAB, thioglycerol) 378 (MH^+) and 400 (MNa^+).

(*Z*)-2-Methoxyimino-3-methyl-4-phenylbut-(*E*)-3-enoic acid 3c. Using general procedure (A), **1a** (0.52 g) was converted into the ester **2c** (0.23 g, 31%). Following general procedure (E), this was then hydrolysed to the *title compound*, obtained as a partially crystalline solid (0.19 g, 77%) (Found: M^+, 219.0892. $C_{12}H_{13}NO_3$ requires *M*, 219.0896); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3450w, 3300–2700br, 1755 and 1720; $\delta_H(CDCl_3)$ 2.16 (3 H, s, CH_3), 4.06 (3 H, s, OCH_3), 6.85 (1 H, br s, =CH), 7.40 (5 H, s, PhH) and 11.04 (1 H, s, CO_2H).

Sodium 6β-[(*Z*)-2-methoxyimino-3-methyl-4-phenylbut-(*E*)-3-enamido]penicillanate 4c. As described in general procedure (G)(i), **3c** (0.18 g) was coupled with 6-APA(TEA). Work-up and purification gave the *title compound* as a colourless, freeze-dried solid (0.17 g, 46%); ν_{max}(KBr)/cm^{-1} 1764, 1661, 1603, 1511, 1399 and 1321; $\delta_H(D_2O)$ 1.50 (3 H, s, 2-CH_3), 1.58 (3 H, s, 2-CH_3), 2.08 (3 H, d, *J* 1.1, CH_3), 3.95 (3 H, s, OCH_3), 4.25 (1 H, s, 3-H), 5.60 and 5.64 (2 H, ABq, *J* 3.9, 5- and 6-H), 6.88 (1 H, d, *J* 1.1, =CH) and 7.35–7.49 (5 H, m, PhH); *m*/*z* (positive ion FAB, thioglycerol) 440 (MH^+) and 462 (MNa^+).

Ethyl (*Z*)-2-cyclopropylmethyloximino-3-oxobutanoate 1d. Ethyl (*Z*)-2-hydroxyimino-3-oxobutanoate[10] (1.59 g) in dry dimethyl sulfoxide (10 cm^{-3}), at room temperature, was treated with cyclopropylmethyl bromide (1.49 g) and potassium carbonate (1.52 g) for 12 h. The reaction mixture was diluted with ethyl acetate, washed with water and brine, dried and concentrated. Chromatography of the residue on silica gel, eluting with 5% ethyl acetate–hexane as eluent afforded the *title compound* as a colourless oil (0.93 g, 44%) (Found:

M^+, 213.1002, $C_{10}H_{15}NO_4$ requires M, 213.1001); ν_{max}-(CH_2Cl_2)/cm^{-1} 1738, 1685, 1595, 1370, 1320 and 1235; δ_H($CDCl_3$) 0.30–0.82 (4 H, m, cyclopropyl H), 1.01–1.35 (1 H, m, cyclopropyl H), 1.33 (3 H, t, J 7.3, CH_2CH_3), 2.73 (3 H, s, $COCH_3$), 4.14 (2 H, d, J 6.7, CH_2) and 4.38 (2 H, q, J 7.3, CH_2CH_3).

Ethyl (*Z*)-2-cyclopropylmethyloximino-3-methylbut-3-enoate 2d. General procedure (A). Ethyl (*Z*)-2-cyclopropylmethyloximino-3-oxobutanoate (2.13 g) was converted into the *title compound* as a colourless oil (0.83 g, 39%) (Found: M^+, 211.1212, $C_{11}H_{17}NO_3$ requires M, 211.1208); ν_{max}-(CH_2Cl_2)/cm^{-1} 1735, 1620w, 1580w and 1450; δ_H($CDCl_3$) 0.30–0.70 (4 H, m, cyclopropyl-H), 0.85–1.20 (1 H, m, cyclopropyl-H), 1.34 (3 H, t, J 7.3, CH_2CH_3), 1.96 (3 H, s, CH_3), 3.98 (2 H, d, J 6.7, CH_2), 4.39 (2 H, q, J 7.3, CH_2CH_3), 5.20 (1 H, br s, =CH) and 5.42 (1 H, m, =CH).

(*Z*)-2-Cyclopropylmethyloximino-3-methylbut-3-enoic acid 3d. Following general procedure (E), hydrolysis of the ester **2d** (0.82 g) gave the *title compound* as a colourless liquid (0.5 g, 70%); ν_{max}(CH_2Cl_2)/cm^{-1} 3650w, 3450w, 1755, 1720, 1620w and 1575; δ_H($CDCl_3$) 0.40–0.72 (4 H, m, cyclopropyl-H), 0.85–1.40 (1 H, m, cyclopropyl-H), 2.01 (3 H, s, CH_3), 4.05 (2 H, d, J 6.7, OCH_2), 5.38 (1 H, br s, =CH), 5.40 (1 H, m, =CH) and 11.00 (1 H, s, CO_2H); m/z (CI, ammonia) 184 (MH^+).

Sodium 6β-[(*Z*)-2-cyclopropylmethyloximino-3-methylbut-3-enamido]penicillanate 4d. Using general procedure (G)(i), coupling of **3d** (0.48 g) with 6-APA(TEA), afforded, after purification, the *title compound* as a pale yellow amorphous solid (0.37 g, 35%); ν_{max}(KBr)/cm^{-1} 1766, 1668, 1603, 1511, 1456, 1399 and 1211; δ_H(D_2O) 0.26–0.32 (2 H, m, cyclopropyl-H), 0.52–0.59 (2 H, m, cyclopropyl-H), 1.13–1.19 (1 H, cyclopropyl-H), 1.52 (3 H, s, 2-CH_3), 1.63 (3 H, s, 2-CH_3), 1.94 (3 H, s, CH_3), 3.98 (2 H, ABq, J 7.2, OCH_2), 4.22 (1 H, s, 3-H), 5.36 (1 H, s, =CH), 5.67 (1 H, d, J 1.3, =CH) and 5.61 and 5.64 (2 H, ABq, J 4.0, 5- and 6-H); m/z (positive ion FAB, thioglycerol) 404 (MH^+), 426 (MNa^+), 807 ($2M + H^+$) and 829 ($2M + Na^+$).

Ethyl (*Z*)-2-cyclohexyloxyimino-3-methylbut-3-enoate 2e. Utilizing general procedure (A), ethyl (*Z*)-2-cyclohexyloxyimino-3-oxobutanoate[1] **1e** (1.5 g) was converted into the *title compound*, a colourless oil (0.8 g, 54%) (Found: M^+, 239.1523, $C_{13}H_{21}NO_3$ requires M, 239.1521); ν_{max}(CH_2Cl_2)/cm^{-1} 1735, 1620w and 1570w; δ_H($CDCl_3$) 1.15–1.95 (13 H, m and t, J 7.3, CH_2CH_3 and cyclohexyl-H), 1.99 (3 H, s, CH_3), 4.05–4.60 (3 H, m and q, J 7.3, OCH and CH_2), 5.22 (1 H, s, =CH) and 5.44 (1 H, m, =CH).

(*Z*)-2-Cyclohexyloxyimino-3-methylbut-3-enoic acid 3e. Compound **2e** (0.8 g) was hydrolysed according to general procedure (E) giving the *title compound* as a colourless gum (0.22 g, 30%). Similarly, general procedure (F) was also used to convert **2e** (1.3 g) into the desired acid **3e** (0.77 g, 66%); ν_{max}(CH_2Cl_2)/cm^{-1} 3650w, 3450w, 1755, 1720 and 1620w; δ_H($CDCl_3$) 1.15–2.10 (10 H, m, cyclohexyl-H), 2.00 (3 H, s, CH_3), 4.02–4.56 (1 H, m, OCH), 5.36 (1 H, s, =CH), 5.49 (1 H, m, =CH) and 10.86 (1 H, s, CO_2H); m/z [positive ion FAB, (3NOBA/Na^+)], 212 (MH^+) and 234 (MNa^+).

Sodium 6β-[(*Z*)-2-cyclohexyloxyimino-3-methylbut-3-enamido]penicillanate 4e. Using general procedure (G)(ii), the acid **3e** (0.77 g) was coupled with 6-APA(TEA). Work-up and purification gave the *title compound* as an amorphous solid (1.2 g, 74%); ν_{max}(KBr)/cm^{-1} 1766, 1668, 1601, 1511, 1450, 1400, 1364 and 1319; δ_H(D_2O) 1.31 (2 H, m, cyclohexyl-H), 1.52 (5 H, s and m, 2-CH_3 and cyclohexyl-H), 1.63 (5 H, s and m, 2-CH_3 and cyclohexyl-H), 1.85 (2 H, m, cyclohexyl-H), 1.94 (3 H, s, CH_3), 4.23 (2 H, s and m, 3-H and OCH), 5.32 (1 H, s, =CH–), 5.54 (1 H, s, =CH–) and 5.60 and 5.65 (2 H, ABq, J 3.9, 5- and 6-H); m/z (positive ion FAB, thioglycerol) 432 (MH^+) and 454 (MNa^+).

Ethyl 4,4-dichloro-(*Z*)-2-methoxyimino-3-methylbut-3-enoate 2f. Ethyl (*Z*)-2-methoxyimino-3-oxobutanoate **1a** (1.73 g), employing general procedure (D) was converted into the *title compound*, a colourless oil (0.64 g, 28%) (Found: M^+, 239.0123, $C_8H_{11}Cl_2NO_3$ requires M, 239.0116); ν_{max}(CH_2Cl_2)/cm^{-1} 1730, 1580, 1460 and 1440; δ_H($CDCl_3$) 1.24 (3 H, t, J 7.3, CH_2CH_3), 2.03 (3 H, s, CH_3), 3.88 (3 H, s, OCH_3) and 4.24 (2 H, q, J 7.3, CH_2CH_3).

4,4-Dichloro-(*Z*)-2-methoxyimino-3-methylbut-3-enoic acid 3f. Following general procedure (E), the ester **2f** (0.62 g) was hydrolysed to give the *title compound* as an oily solid (0.53 g, 96%); ν_{max}(CH_2Cl_2)/cm^{-1} 3450w, 3300–2700br, 1755 and 1620; δ_H($CDCl_3$) 2.12 (3 H, s, CH_3), 4.03 (3 H, s, OCH_3) and 11.40 (1 H, s, CO_2H); m/z (CI) 229 (MNH_4^+).

Sodium 6β-[4,4-dichloro-(*Z*)-2-methoxyimino-3-methylbut-3-enamido]penicillanate 4f. Employing general procedure (G)(i), the acid **3f** (0.52 g) was coupled with 6-APA(TEA). Following purification, the *title compound* was obtained as a colourless, amorphous solid (0.47 g, 45%); ν_{max}(KBr)/cm^{-1} 1766, 1668, 1608, 1516, 1400 and 1321; δ_H(D_2O) 1.52 (3 H, s, 2-CH_3), 1.64 (3 H, s, 2-CH_3), 2.11 (3 H, s, CH_3), 4.00 (3 H, s, OCH_3), 4.26 (1 H, s, 3-H) and 5.60 and 5.62 (2 H, ABq, J 4.1, 5- and 6-H); m/z (positive ion FAB, thioglycerol) 432 (MH^+), 454 (MNa^+) and 885 ($2M + Na^+$).

Ethyl (*Z*)-2-*tert*-butoxyimino-4,4-dichloro-3-methylbut-3-enoate 2g. The modification of general procedure (D), was used to convert ethyl (*Z*)-2-*tert*-butoxyimino-3-oxobutanoate[1] **1g** (2.15 g) into the *title compound* as a colourless oil (0.38 g, 13%) (Found: M^+, 281.0584, $C_{11}H_{17}Cl_2NO_3$ requires M, 281.0585); ν_{max}(CH_2Cl_2)/cm^{-1} 1740, 1590w and 1370; δ_H($CDCl_3$) 1.30 [9 H, s, $C(CH_3)_3$], 1.35 (3 H, t, J 7.3, CH_2CH_3), 2.14 (3 H, s, CH_3) and 4.35 (2 H, q, J 7.3, CH_2).

(*Z*)-2-*tert*-Butoxyimino-4,4-dichloro-3-methylbut-3-enoic acid 3g. Compound **2g** (0.35 g) was hydrolysed as outlined in general procedure (F) to give the *title compound* as a semi-solid (0.31 g, 98%); ν_{max}(CH_2Cl_2)/cm^{-1} 3450w, 3200br, 1750, 1620w, 1380 and 1360; δ_H($CDCl_3$) 1.38 [9 H, s, $C(CH_3)_3$], 2.12 (3 H, s, CH_3) and 11.18 (1 H, s, CO_2H); m/z (EI) 254 (MH^+).

Sodium 6β-[(*Z*)-2-*tert*-butoxyimino-4,4-dichloro-3-methylbut-3-enamido]penicillanate 4g. Utilizing general procedure (G)(ii), the acid **3g** (0.31 g) was coupled to 6-APA(TEA). Purification afforded the title compound as an amorphous white solid (0.27 g, 54%); ν_{max}(KBr)/cm^{-1} 1779, 1665, 1599, 1507, 1453 and 1403; δ_H(D_2O) 1.32 [9 H, s, $C(CH_3)_3$], 1.49 (3 H, s, 2-CH_3), 1.61 (3 H, s, 2-CH_3), 2.06 (3 H, s, CH_3), 4.21 (1 H, s, 3-H) and 5.58 and 5.63 (2 H, ABq, J 4.0, 5- and 6-H); m/z (positive ion FAB, thioglycerol) 347 (MH^+).

2-Aminothiazol-4-ylmethyl(triphenyl)phosphonium chloride 6. 2-Amino-4-chloromethyltriazole hydrochloride[4] **5** (17 g), as a suspension in ethanol (750 cm^3) and triphenylphosphine (24.3 g) were heated under reflux for 2 days after which the mixture was allowed to cool when a white precipitate was formed. The mixture was filtered and the filtrate evaporated under reduced pressure. The residue was dissolved in water (500 cm^3) and the solution extracted with ethyl acetate. The aqueous phase was adjusted to pH 7.5 with dilute aqueous sodium hydroxide and, when stored, the *title compound* crystallized from it. The product was filtered off, dried over phosphorus pentoxide *in vacuo* to give a white solid (21 g, 56%), mp 251–252 °C (decomp. ethanol) (Found: C, 64.3; H, 5.3; N, 6.9. $C_{22}H_{20}ClN_2PS$ requires C, 64.31; H, 4.91; N, 6.82%); δ_H[$(CD_3)_2SO$–D_2O] 4.86 (2 H, d, J 14.0, CH_2), 6.30 (1 H, d, J 4.0, 5-H) and 7.67–7.80 (15 H, m, PhH).

Ethyl 4-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyimino-3-methyl-(*E*)-but-3-enoate 7a. As described in general procedure (B), initially at room temperature and then at 80 °C, the ester **1a** (0.87 g) was converted into the *title compound* (0.37 g, 28%), although the starting ester was recovered in 68% yield.

Conversion of **1a** (0.04 g) was also achieved by using general procedure (C) (0.03 g, 41%), mp 105–106 °C (ethyl acetate–hexane) (Found: C, 49.2; H, 5.7; N, 15.5; S, 11.9%; M^+, 269.0847. $C_{11}H_{15}N_3O_3S$ requires C, 49.06; H, 5.61; N, 15.60; S, 11.91%; *M*, 269.0835); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3500, 3400, 1735, 1605 and 1530; $\delta_H(CDCl_3)$ 1.34 (3 H, t, *J* 7.3, CH_2CH_3), 2.27 (3 H, br s, CH_3), 3.96 (3 H, s, OCH_3), 4.39 (2 H, q, *J* 7.3, CH_2CH_3), 5.47 (2 H, br s, NH_2), 6.35 (1 H, br s, =CH) and 6.56 (1 H, s, 5-H).

4-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyimino-3-methyl-(*E*)-but-3-enoic acid 8a. As outlined in general procedure (E), the ester **7a** (0.3 g) was hydrolysed to give the title compound as an amorphous white solid (0.24 g, 89%), mp 206–207 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3486, 3326br, 1626, 1600, 1583, 1560 and 1408; $\delta_H[(CD_3)_2SO]$ 2.24 (3 H, s, CH_3), 4.83 (3 H, s, OCH_3), 6.23 (1 H, s, =CH), 6.77 (1 H, s, =CH) and 7.00 (3 H, br s, NH_2 and CO_2H, exchangeable); *m/z* (EI) 241 (M^+).

Sodium 6β-[4-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyimino-3-methyl-(*E*)-but-3-enamido]penicillanate 9a. The acid **8a** (0.2 g) was coupled to 6-APA(TEA) as detailed in general procedure (G)(iii). In this case, following HP20SS chromatography, the *N*,*N*-diisopropylethylammonium salt (0.09 g, 19%) was isolated (by ^1H NMR). A solution of this salt in water (2 cm^3), was converted into the title compound by eluting through a column of Amberlite IR-120 (Na^+) resin and freeze-drying the eluent. The product was thus obtained as an amorphous white solid (0.07 g, 18%); ν_{max}(KBr)/cm $^{-1}$ 1773, 1672, 1610, 1529 and 1460; $\delta_H(D_2O)$ 1.54 (3 H, s, 2-CH_3), 1.60 (3 H, s, 2-CH_3), 2.06 (3 H, s, CH_3), 3.95 (3 H, s, OCH_3), 4.26 (1 H, s, 3-H), 5.66 (2 H, s, 5- and 6-H), 6.58 (1 H, br s, =CH) and 6.77 (1 H, s, aminothiazole 5-H); *m/z* (positive ion FAB, thioglycerol) 400 (MH^+).

Ethyl 4-(2-aminothiazol-4-yl)-(*Z*)-2-cyclohexyloximino-3-methyl-(*E*)-but-3-enoate 7b. Using general procedure (C) at room temperature, ethyl (*Z*)-2-cyclohexyloxyimino-3-oxobutanoate **1e** (2 g) was converted into the *title compound*, a colourless oil (0.73 g, 26%) (Found: M^+, 337.1458. $C_{16}H_{23}N_3O_3S$ requires *M*, 337.1460); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3500, 3400, 1735, 1610 and 1530; $\delta_H(CDCl_3)$ 1.25–2.00 (13 H, m, cyclohexyl-H and CH_2CH_3), 2.30 (3 H, br s, CH_3), 4.12–4.61 (3 H, m, OCH and CH_2CH_3), 5.50 (2 H, br s, NH_2), 6.40 (1 H, br s, =CH) and 6.57 (1 H, s, 5-H).

4-(2-Aminothiazol-4-yl)-(*Z*)-2-cyclohexyloxyimino-3-methyl-(*E*)-but-3-enoic acid 8b. Since the ester **7b** (0.68 g) was only partially hydrolysed using general procedure (E), the recovered starting material was then fully hydrolysed using general procedure (F). The combined products gave the *title compound* as an amorphous white solid (0.27 g, 43%) (Found: M^+, 309.1165, $C_{14}H_{19}N_3O_3S$ requires *M*, 309.1146); ν_{max}(KBr)/cm^{-1} 3285br, 1643, 1610, 1583, 1560 and 1451; $\delta_H[(CD_3)_2SO]$ 1.28–1.46 (6 H, m, cyclohexyl-H), 1.64 (2 H, m, cyclohexyl-H), 1.81 (2 H, m, cyclohexyl-H), 2.28 (3 H, s, CH_3), 4.06 (1 H, m, OCH), 6.18 (1 H, s, =CH), 6.77 (1 H, s, 5-H) and 7.03 (2 H, s, NH_2, exchangeable).

Sodium 6β-[4-(2-aminothiazol-4-yl)-(*Z*)-2-cyclohexyloxyimino-3-methyl-(*E*)-but-3-enamido]penicillanate 9b. The acid **8b** (0.25 g), was coupled to 6-APA(TEA) according to general procedure (G)(iii) to give the title compound as an amorphous solid (0.25 g, 57%); ν_{max}(KBr)/cm^{-1} 1771, 1670, 1609, 1523, 1451 and 1399; $\delta_H(D_2O)$ 1.29 (3 H, m, cyclohexyl-H), 1.49 (6 H, s and m, 2-CH_3 and cyclohexyl-H), 1.57 (5 H, s and m, 2-CH_3 and cyclohexyl-H), 1.84 (2 H, m, cyclohexyl-H), 2.04 (3 H, s, CH_3), 4.21 (2 H, s and m, 3-H and OCH), 5.61 and 5.65 (2 H, ABq, *J* 4.0, 5- and 6-H), 6.50 (1 H, s, =CH–) and 6.72 (1 H, s, aminothiazole 5-H); *m/z* (positive ion FAB, thioglycerol) 530 (MH^+) and 552 (MNa^+).

Ethyl 4-(2-aminothiazol-4-yl)-(*Z*)-2-*tert*-butoxyimino-3-methyl-(*E*)-but-3-enoate 7c. Employing general procedure (C) at room temperature, ethyl (*Z*)-2-*tert*-butoxyimino-3-oxobutanoate **1g** (1.95 g) was converted into the *title compound* (0.40 g, 14%) (Found: M^+, 311.1294. $C_{14}H_{21}N_3O_3S$ requires *M*, 311.1302); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3475w, 3380w, 1725, 1600 and 1525; $\delta_H(CDCl_3)$, 1.30 [12 H, s and t, *J* 7.3, $C(CH_3)_3$ and CH_2CH_3], 2.30 (3 H, br s, CH_3), 4.41 (2 H, q, *J* 7.3, CH_2CH_3), 5.40 (2 H, br s, NH_2, exchangeable), 6.38 (1 H, br s, =CH) and 6.56 (1 H, s, 5-H).

4-(2-Aminothiazol-4-yl)-(*Z*)-2-*tert*-butoxyimino-3-methylbut-3-enoic acid 8c. Adopting general procedure (F), the ester **7c** (0.39 g) was converted into the *title compound*, an amorphous solid (0.21 g, 60%) (Found: M^+, 283.0991. $C_{12}H_{17}N_3O_3S$ requires *M*, 283.0989); ν_{max}(KBr)/cm^{-1} 3279br, 1641, 1602 and 1472; $\delta_H[(CD_3)_2CO$–$(CD_3)_3SO]$ 1.30 [9 H, s, $C(CH_3)_3$], 2.36 (3 H, s, CH_3), 6.46 (1 H, br s, =CH), 6.58 (2 H, br s, NH_2, exchangeable) and 6.75 (1 H, s, 5-H).

Sodium 6β-[4-(2-aminothiazol-4-yl)-(*Z*)-2-*tert*-butoxyimino-3-methyl-(*E*)-but-3-enamido]penicillanate 9c. As described in general procedure (G)(iii), the acid **8c** (0.19 g) was coupled to 6-APA(TEA) to give the title compound as an amorphous white solid (0.21 g, 61%); ν_{max}(KBr)/cm^{-1} 1769, 1669, 1609, 1526 and 1458; $\delta_H(D_2O)$ 1.30 [9 H, s, $C(CH_3)_3$], 1.50 (3 H, s, 2-CH_3), 1.59 (3 H, s, 2-CH_3), 2.18 (3 H, s, CH_3), 4.21 (1 H, s, 3-H), 5.61 and 5.65 (2 H, ABq, *J* 4.0, 5- and 6-H), 6.44 (1 H, s, =CH) and 6.75 (1 H, s, aminothiazole 5-H); *m/z* (positive ion FAB, thioglycerol) 504 (MH^+) and 526 (MNa^+).

Sodium 7β-[4-(2-aminothiazol-4-yl)-(*Z*)-2-*tert*-butoxyimino-3-methyl-(*E*)-but-3-enamido]cephalosporanate 9d. Using the same general procedure (G)(iii), the acid **8a** (0.22 g) was coupled to triethylammonium 7β-aminocephalosporanate [7-ACA-(TEA)]. Following the same isolation and purification methods, the title compound was obtained as a white, freeze-dried solid (0.16 g, 34%); ν_{max}(KBr)/cm^{-1} 1763, 1670, 1611, 1527, 1396, 1351 and 1234; $\delta_H(D_2O)$ 2.05 (3 H, s, CH_3), 2.10 (3 H, s, $OCOCH_3$), 3.37 and 3.66 (2 H, ABq, *J* 18.0, SCH_2), 3.93 (3 H, s, OCH_3), 4.67 and 4.85 (2 H, ABq, *J* 12.4, CH_2O), 5.18 (1 H, d, *J* 4.6, 6-H), 5.81 (1 H, d, *J* 4.8, 7-H), 6.46 (1 H, s, =CH) and 6.77 (1 H, s, aminothiazole 5-H); *m/z* (positive ion FAB, thioglycerol) 518 (MH^+) and 540 (MNa^+).

Ethyl (*E*)-2-hydroxyimino-3-oxo-3-phenylpropanoate 11. Ethyl benzoylacetate **10** (11.52 g) in glacial acetic acid (60 cm^3), was cooled to *ca.* 5 °C and treated with a solution of sodium nitrite (4.8 g) in water (12 cm^3) in a dropwise fashion such that the temperature was maintained < 10 °C. After 30 min at room temperature the solution was poured into water and stirred for 10 min. After this, the product was filtered off, washed with water and dried over phosphorus pentoxide to give a white solid (9.93 g, 75%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3530, 3300br, 1745, 1735, 1685, 1600w and 1585; $\delta_H(CDCl_3)$ 1.21 (3 H, t, *J* 7.3, CH_2CH_3), 4.29 (2 H, q, *J* 7.3, CH_2CH_3) and 7.30–8.00 (5 H, m, PhH).

(*E*)-2-Hydroxyimino-3-oxo-3-phenylpropanoic acid 12. The ester **11** (1 g) was hydrolysed as described in general procedure (E) to give the product as an amorphous solid (0.76 g, 87%); ν_{max}(Nujol)/cm^{-1} 3320br, 1720, 1680, 1620w, 1595w, 1580w and 1450; $\delta_H[(CD_3)_2CO]$ 7.66–8.09 (7 H, m, overlapping vbr s, PhH and OH).

Confirmation of the *E* stereochemistry of compounds 11 and 12; preparation of benzoylnitrile.[11] A suspension of the acid **12** (0.21 g) in dichloromethane (5 cm^3) was treated with *N*,*N*-dimethylaminopyridine (0.26 g) after which slow addition of acetyl chloride (0.15 cm^3) resulted in darkening of the solution and evolution of a gas. After 30 min the homogeneous solution was concentrated and partitioned between ethyl acetate and water. After washing and drying of the organic phase, it was evaporated under reduced pressure to afford a dark-brown semi-solid (0.1 g, 70%); the IR spectrum confirmed the presence of the nitrile moiety: $\nu_{max}(CH_2Cl_2)$/cm $^{-1}$ 2225, 1815w, 1680, 1600 and 1450; $\delta_H(CDCl_3)$ 7.52–8.33 (5 H, m, PhH).

Ethyl (*Z*)-2-hydroxyimino-3-oxo-3-phenylpropanoate 16. The *E*-isomer **11** (1.92 g) in dry benzene (150 cm^3) and 5% palladium-on-alumina, was heated under reflux for 24 h, after which the reaction mixture was filtered through Kieselguhr, concentrated and purified by silica gel chromatography. Elution with 20% ethyl acetate–hexane gave the *title compound* as a yellow oil (0.62 g, 32%) (Found: M^+, 221.0686. $C_{11}H_{11}NO_4$ requires *M*, 221.0687); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3530, 3350br, 1745, 1660, 1600w, 1580w, 1450w and 1370; $\delta_H(CDCl_3)$ 1.33 (3 H, t, *J* 7.3, CH_2CH_3), 4.44 (2 H, q, *J* 7.3, CH_2CH_3) and 7.32–8.15 (5 H, m, PhH).

Ethyl (*Z*)-2-methoxyimino-3-oxo-3-phenylpropanoate 17. The hydroxyimino compound **16** (0.57 g) in dry dimethyl sulfoxide (2 cm^3) was treated with potassium carbonate (0.53 g) and iodomethane (0.24 cm^3) at room temperature for 30 min after which the reaction mixture was poured into water (20 cm^3) and extracted with ethyl acetate (2 × 20 cm^3). The organic phase was washed with water and brine, dried and evaporated to give the crude product. Purification of this by silica gel chromatography eluting with 20% ethyl acetate–hexane afforded the title compound as a colourless liquid (0.45 g, 75%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1740, 1660, 1600, 1580w and 1445; $\delta_H(CDCl_3)$ 1.36 (3 H, t, *J* 7.3, CH_2CH_3), 4.15 (3 H, s, OCH_3), 4.44 (2 H, q, *J* 7.3, CH_2CH_3), 7.62 (3 H, m, PhH) and 8.13 (2 H, m, PhH).

Ethyl 4,4-dichloro-(*Z*)-2-methoxyimino-3-phenylbut-3-enoate 18. The ester **17** (0.45 g) was converted into the product according to the modified general procedure (D). After purification the *title compound* was obtained as a colourless oil (0.55 g, 94%) (Found: M^+, 301.0278, $C_{13}H_{13}Cl_2NO_3$ requires *M*, 301.0273); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1740, 1590w and 1560w; $\delta_H(CDCl_3)$ 1.23 (3 H, t, *J* 7.3, CH_2CH_3), 3.97 (3 H, s, OCH_3), 4.28 (2 H, q, *J* 7.3, CH_2CH_3) and 7.40 (5 H, s, PhH).

4,4-Dichloro-(*Z*)-2-methoxyimino-3-phenylbut-3-enoic acid 19. As outlined in general procedure (E), the ester **18** (0.54 g) was hydrolysed to give the *title compound* as a colourless gum (0.49 g, 100%) (Found: M^+, 272.9957, $C_{11}H_9Cl_2NO_3$ requires *M*, 272.9960); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3470w, 3300w and 1755; $\delta_H(CDCl_3)$ 4.04 (3 H, s, OCH_3), 7.40 (5 H, s, PhH) and 11.92 (1 H, s, CO_2H).

6β-[4,4-Dichloro-(*Z*)-2-methoxyimino-3-phenylbut-3-enamido]penicillanic acid 20. As described in general procedure (G)(iii), the acid **19** (0.47 g) was coupled to 6-APA(TEA). In this case, however, elution of the crude sodium salt through HP20SS resulted in isolation of the free acid, extracted from the combined aqueous fractions with ethyl acetate. Evaporation of the solvent afforded the *title compound* as a pale yellow foam (0.28 g, 35%); $\nu_{max}(KBr)/cm^{-1}$ 1786, 1743, 1671 and 1516; $\delta_H(CDCl_3)$ 1.59 (3 H, s, 2-CH_3), 1.67 (3 H, s, 2-CH_3), 4.12 (3 H, s, OCH_3), 4.48 (1 H, s, 3-H), 5.54 (1 H, d, *J* 4.2, 5-H), 5.72 (1 H, dd, *J* 4.2, 8.7, 6-H), 7.31–7.44 (5 H, m, PhH) and 8.18 (1 H, d, *J* 8.7, NH); *m/z* (positive ion FAB, thioglycerol) 472 (MH^+).

Ethyl (*Z*)-2-methoxyimino-3-oxopentanoate 23. Ethyl 3-oxopentanoate (1.44 g) was converted into the (*Z*)-hydroxyimino compound in acetic acid (5 cm^3) with sodium nitrite (0.76 g) in 79% yield as previously described for compound **11**. The crude product was methylated in dry dimethyl sulfoxide (5 cm^3) with iodomethane (0.74 cm^3) and potassium carbonate (1.63 g) as outlined for compound **17**. Following work-up and purification by silica gel chromatography eluting with 5% ethyl acetate–hexane, the *title compound* was isolated as a colourless liquid (1.26 g, 86%) (Found: M^+, 187.0845, $C_8H_{13}NO_4$ requires *M*, 187.0844); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1740, 1695, 1600w, 1460w and 1370; $\delta_H(CDCl_3)$ 1.14 (3 H, t, *J* 7.3, CH_2CH_3), 1.36 (3 H, t, *J* 7.3, $CO_2CH_2CH_3$), 2.84 (2 H, q, *J* 7.3, CH_2CH_3), 4.13 (3 H, s, OCH_3) and 4.49 (2 H, q, *J* 7.3, $CO_2CH_2CH_3$).

Ethyl 4-bromo-(*Z*)-2-methoxyimino-3-oxopentanoate 24. The ester **23** (1 g) in carbon tetrachloride (10 cm^3), at room temperature, containing hydrogen bromide–acetic acid (45% w/w solution; 0.96 cm^3) was treated dropwise with a solution of bromine (0.3 cm^3) in carbon tetrachloride (10 cm^3). After complete addition, the red solution was poured into water (50 cm^3). The organic phase was washed with saturated aqueous sodium thiosulfate (50 cm^3) and brine (50 cm^3), dried and evaporated to afford the unstable title compound as a pale yellow oil (1.35 g, 95%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1740, 1700, 1600w, 1445w and 1370; $\delta_H(CDCl_3)$ 1.37 (3 H, t, *J* 7.3, $CO_2CH_2CH_3$), 1.83 (3 H, d, *J* 7.0, $CHCH_3$), 4.20 (3 H, s, OCH_3), 4.40 (2 H, q, *J* 7.3, $CO_2CH_2CH_3$) and 5.30 (1 H, q, *J* 7.0, CHBr); *m/z* (EI) 234 ($M^+ - OCH_3$).

Ethyl 3,4-dioxo-(*Z*)-2-methoxyiminopentanoate 21. The bromide **24** (3.64 g) in dry acetonitrile (10 cm^3), was treated with a solution of silver nitrate (2.9 g) in acetonitrile (10 cm^3). After 24 h, the precipitate was filtered off and the solution concentrated. The crude nitrate **25** (3.28 g) was taken up in dry dimethyl sulfoxide (10 cm^3) and treated with sodium acetate trihydrate (0.19 g) to give an intense yellow solution. After 15 min the reaction mixture was poured into water (50 cm^3) and extracted with ethyl acetate. The extract was washed with water and brine, dried and concentrated to give the crude product which was purified by silica gel chromatography. The title compound was obtained as a yellow liquid (1.63 g, 59%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1745, 1695 and 1590w; $\delta_H(CDCl_3)$ 1.35 (3 H, t, *J* 7.3, CH_2CH_3), 2.42 (3 H, s, CH_3CO), 4.19 (3 H, s, OCH_3) and 4.42 (2 H, q, *J* 7.3, CH_2CH_3); *m/z* (EI) 201 (M^+).

Ethyl 3-(2-aminothiazol-4-yl)-2-methoxyimino-3-oxopropanoate 22. The diketone **21** (6.46 g) and triethylamine (5.59 cm^3), in dry dichloromethane (50 cm^3), under argon, was treated with trimethylsilyl trifluoromethanesulfonate (7.76 cm^3) with cooling. After 1 h, *N*-bromosuccinimide (6.29 g) was added to the mixture. After a further 1 h at room temperature the mixture was washed with water, saturated aqueous sodium thiosulfate and brine, dried and evaporated under reduced pressure to give the crude bromide **26**. This was redissolved in ethanol (25 cm^3) and treated with thiourea (3.05 g) and *N*,*N*-dimethylaniline (5.08 cm^3) overnight. Solvent was removed from the mixture under reduced pressure and the residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with water and brine and then dried and evaporated. Silica gel chromatography of the residue afforded the *title compound* as a mixture of isomers (1.94 g, 23%). The yellow gum was taken up in ether and triturated to afford the product, a yellow solid, as a single isomer (0.39 g, 5%), mp 127–128.5 °C (ethyl acetate) (Found: C, 42.3; H, 4.3; N, 16.0; S, 12.5. $C_9H_{11}N_3O_4S$ requires C, 42.02; H, 4.31; N, 16.33; S, 12.46%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3470w, 3380w, 1740, 1650, 1605, 1535; $\delta_H(CDCl_3)$ 1.38 (3 H, t, *J* 7.3, CH_2CH_3), 4.19 (3 H, s, OCH_3), 4.44 (2 H, q, *J* 7.3, CH_2CH_3) 7.94 (1 H, s, aminothiazole 5-H).

Ethyl 4,4-dichloro-3-ethyl-(*Z*)-2-methoxyiminobut-3-enoate 27. The ester **23** (10.84 g) was converted into the title compound by the method employed in general procedure (D). After purification by silica gel chromatography the *title compound* was isolated as a yellow liquid (7.68 g, 52%) (Found: M^+, 253.0282, $C_9H_{13}Cl_2NO_3$ requires *M*, 253.0272); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1725, 1600w, 1570w and 1460; $\delta_H(CDCl_3)$ 1.10 (3 H, t, *J* 7.3, CH_2CH_3), 1.33 (3 H, t, *J* 7.3, $CO_2CH_2CH_3$), 2.59 (2 H, q, *J* 7.3, CH_2CH_3), 4.00 (3 H, s, OCH_3) and 4.25 (2 H, q, *J* 7.3, $CO_2CH_2CH_3$).

Ethyl 3-(1-bromoethyl)-4,4-dichloro-(*Z*)-2-methoxyiminobut-3-enoate 28. The ester **27** (5 g) in carbon tetrachloride (100 cm^3) with *N*-bromosuccinimide (4.2 g) and a trace of azoisobutyronitrile was heated under reflux for 15 min. The succinimide was filtered off, the filtrate evaporated and the residue purified by silica gel chromatography, eluting with 5% ethyl acetate–hexane, to give the *title compound* as a colourless oil (5.59 g, 85%) (Found: M^+, 329.9299, $C_9H_{11}BrCl_2NO_3$ requires *M*,

329.9300); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1730 and 1580; $\delta_H(CDCl_3)$ 1.35 (3 H, t, *J* 7.3, CH_2CH_3), 1.85 (3 H, d, *J* 7.3, CH_3CH), 4.09 (3 H, s, OCH_3), 4.37 (2 H, q, *J* 7.3, CH_2CH_3) and 5.36 (1 H, q, *J* 7.3, CHBr).

4-Dichloromethylidene-(*Z*)-3-methoxyimino-5-methyl-2-oxotetrahydrofuran 30. The bromide **28** (0.3 g) in acetonitrile (5 cm^3) was treated at room temperature with silver trifluoroacetate (0.6 g). After 30 min, the grey–green precipitate was removed by filtration through Celite and the filtrate evaporated under reduced pressure. The residue was dissolved in ether and the solution washed with water and brine, dried and evaporated. The crude product was dissolved in 50% aqueous methanol (5 cm^3), and the solution adjusted to pH 7 at which value it was maintained by the dropwise addition of saturated aqueous sodium hydrogen carbonate. After the methanol had been evaporated the aqueous solution was extracted with ether. After concentration of the extract the crude product was purified by silica gel chromatography, eluting with 10% ethyl acetate–hexane to give the *title compound* as a colourless crystalline solid (0.15 g, 73%), mp 70–71 °C (cyclohexane) (Found: C, 37.2; H, 3.1; N, 6.2; S, 31.8%; M^+, 222.9799. $C_7H_7Cl_2NO_3$ requires C, 37.53; H, 3.15; N, 6.25; S, 31.65%; *M*, 222.9803); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1780, 1730sh, 1610 and 1560; $\delta_H(CDCl_3)$ 1.60 (3 H, d, *J* 6.7, CH_3), 4.25 (3 H, s, OCH_3) and 5.27 (1 H, q, *J* 6.7, CH).

Methyl 3-acetyl-4,4-dichloro-(*Z*)-2-methoxyiminobut-3-enoate 32. The lactone **30** (0.51 g) was brominated with *N*-bromosuccinimide (0.49 g) in carbon tetrachloride (5 cm^3) as described for compound **28**. Isolation of the crude bromide **31**, was followed by its re-dissolution in acetone (5 cm^3) and treatment with an excess of saturated aqueous sodium hydrogen carbonate for 15 min at room temperature. After the initial effervescence had subsided, acetone was removed from the mixture by evaporation and the aqueous solution was extracted with ethyl acetate. After acidification of the aqueous solution with dilute hydrochloric acid it was re-extracted with ethyl acetate. The extract was washed with brine, dried and concentrated to give a yellow gum. This was dissolved in DMF (2 cm^3) and treated with potassium carbonate (0.46 g) and iodomethane (0.21 cm^3). After 1 h, the reaction mixture was diluted with ethyl acetate, washed with water (3 ×) and brine, dried and concentrated. Silica gel chromatography of the residue afforded the *title compound* as a colourless oil (0.27 g, 46%) (Found: M^+, 252.9905, $C_8H_9Cl_2NO_4$ requires *M*, 252.9909); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1740br and 1570; $\delta_H(CDCl_3)$ 2.40 (3 H, s, CH_3CO), 3.87 (3 H, s, CO_2CH_3) and 3.95 (3 H, s, OCH_3).

Methyl 3-(2-aminothiazol-4-yl)-4,4-dichloro-(*Z*)-2-methoxyiminobut-3-enoate 34. Utilizing the method described for compound **22**, the ketone **35** (0.25 g) in dichloromethane (5 cm^3) with triethylamine (0.21 cm^3) and trimethylsilyl trifluoromethanesulfonate (0.29 cm^3) was first converted into the α-bromo ketone **33**. The crude bromide in ethanol (5 cm^3) was treated with thiourea (0.11 g) and *N*,*N*-dimethylaniline (0.19 cm^3) overnight at room temperature after which solvent removal under reduced pressure followed by silica gel chromatography afforded the *title compound* as a yellow crystalline solid (0.17 g, 55%), mp 146–147 °C (ethyl acetate–hexane) (Found: C, 35.2; H, 2.9; Cl, 22.6; N, 13.1; S, 10.3%; M^+, 308.9747. $C_9H_9Cl_2N_3O_3S$ requires C, 34.85; H, 2.92; Cl, 22.86; N, 13.55; S, 10.34%; *M*, 308.9742); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3470w, 3375w, 1730, 1600 and 1520; $\delta_H(CDCl_3)$ 3.80 (3 H, s, CO_2CH_3), 4.04 (3 H, s, OCH_3), 5.52 (2 H, br s, NH_2) and 6.85 (1 H, s, aminothiazole 5-H).

Methyl-3-(2-chloroacetamidothiazol-4-yl)-4,4-dichloro-(*Z*)-2-methoxyiminobut-3-enoate 35. The aminothiazole derivative **34** (0.5 g) as a suspension in dichloromethane (20 cm^3) with triethylamine (0.34 cm^3) cooled to 0 °C was treated with a solution of chloroacetyl chloride (0.19 cm^3) in dichloromethane (5 cm^3) over 15 min. The homogeneous solution was left at room temperature over 48 h after which it was evaporated under reduced pressure and the residue chromatographed on silica gel, eluting with 20% ethyl acetate–hexane to give the *title compound* as a colourless crystalline solid (0.58 g, 92%), mp 136–137 °C (ethyl acetate–hexane) (Found: C, 34.5; H, 2.6; Cl, 27.6; N, 10.7; S, 8.14%; M^+, 384.9459. $C_{11}H_{10}Cl_3N_3O_4S$ requires C, 34.17; H, 2.61; Cl, 27.51; N, 10.87; S, 8.29%; *M*, 384.9458); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3350w, 1735, 1685 and 1530; $\delta_H(CDCl_3)$ 3.80 (3 H, s, CO_2CH_3), 3.99 (3 H, s, OCH_3), 4.28 (2 H, s, $ClCH_2CO$) and 7.34 (1 H, s, aminothiazole 5-H).

3-(2-Chloroacetamidothiazol-4-yl)-4,4-dichloro-(*Z*)-2-methoxyiminobut-3-enoic acid 36. The ester **35** (0.5 g) in methanol (5 cm^3) was hydrolysed and isolated according to general procedure (E). The *title compound*, obtained as a colourless foam, was crystallized from ethyl acetate–hexane (0.42 g, 88%), mp 136–137 °C (ethyl acetate–hexane) (Found: C, 32.5; H, 2.2; N, Cl, 28.1; 11.05; S, 9.0. $C_{10}H_8Cl_3N_3O_4S$ requires C, 32.23; H, 2.16; Cl, 28.55; N, 11.28; S, 8.61%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3370w, 1750, 1690 and 1535; $\delta_H(CDCl_3)$ 4.07 (3 H, s, OCH_3), 4.30 (2 H, s, $ClCH_2CO$), 7.42 (1 H, s, aminothiazole 5-H) and 10.76 (1 H, br s, CO_2H); *m*/*z* (positive ion FAB, thioglycerol) 372 (MH^+).

Sodium 6β-[3-(2-aminothiazol-4-yl)-4,4-dichloro-(*Z*)-2-methoxyiminobut-3-enamido]penicillanate 37. Using general procedure (G)(iii), the acid **36** (0.38 g) was coupled to 6-APA-(TEA) to give an intense yellow precipitate. After 30 min, this was filtered off, washed with dichloromethane and dried. The filtrate was diluted with dichloromethane, washed with dilute hydrochloric acid and brine, dried and evaporated to give the crude penicillanic acid as a colourless foam (0.22 g). This was re-dissolved in 50% aqueous THF (5 cm^3) and treated with sodium *N*-methyldithiocarbamate (0.1 g) overnight at room temperature. The solvent was removed from the mixture under reduced pressure and the residue dissolved in saturated aqueous sodium hydrogen carbonate and purified as previously described. The title compound was obtained as an amorphous white solid (0.09 g, 17%); $\nu_{max}(KBr)/cm^{-1}$ 1766, 1664, 1610, 1526, 1458w, 1400 and 1323; $\delta_H(D_2O)$ 1.48 (3 H, s, 2-CH_3), 1.55 (3 H, s, 2-CH_3), 4.01 (3 H, s, OCH_3), 4.22 (1 H, s, 3-H), 5.50 and 5.55 (2 H, ABq, *J* 4.0, 5- and 6-H) and 7.02 (1 H, s, aminothiazole-H); *m*/*z* (positive ion FAB, thioglycerol) 516 (MH^+) and 538 (MNa^+).

The solid (0.29 g), shown to be the bicyclic compound **38** (Found: M^+, 352.9188, $C_{10}H_6Cl_3N_3O_3S$ requires *M*, 352.9195), was re-suspended in dichloromethane (10 cm^3) and treated with an excess of 6-APA(TEA) at room temperature to give a colourless homogeneous solution after 30 min. TLC (7:2:1, ethyl acetate–butanol–water) showed clean formation of the triethylammonium salt of **37** which was compared to an authentic sample. Work-up and isolation provided a second crop of the product.

Methyl 2-(1-hydroxycyclohexyl)-2,2-dimethoxyacetate 39a. Lithium diisopropylamide was prepared *in situ* from diisopropylamine (10.5 cm^3) and butyllithium (1.6 mol dm^{-3} solution in hexane; 46 cm^3) in dry THF (75 cm^3), under argon at −78 °C and methyl dimethoxyacetate[7] (9.17 cm^3) in THF (75 cm^3) was added dropwise to it over 15 min. After a further 15 min, cyclohexanone (4.9 g) in THF (60 cm^3) was added over 15 min to the reaction mixture which was then maintained at −78 °C for 30 min before being poured into water (200 cm^3). The aqueous phase was extracted with ethyl acetate (100 cm^3) and the extract washed with water and brine, dried and evaporated under reduced pressure. Silica gel chromatography of the residue eluting with 10% ethyl acetate–hexane gave the *title compound* as a pale yellow, waxy solid (7.78 g, 67%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3550, 1740 and 1435; $\delta_H(CDCl_3)$ 1.10–1.95 (10 H, m, cyclohexyl-H), 2.42 (1 H, s, OH), 3.48 (6 H, s, OCH_3) and 3.83 (3 H, s, CO_2CH_3); *m*/*z* (CI) 250 (MNH_4^+).

Methyl 2-(cyclohex-1-enyl)-2-oxoacetate 40a. The hydroxy compound **39a** (7.41 g) in dichloromethane (300 cm^3) was treated with pyridine (24.4 cm^3), and the mixture cooled to 0 °C when thionyl chloride (9.15 cm^3) was slowly added to it. After 1 h the mixture was evaporated under reduced pressure and the residue taken up in dioxane (200 cm^3) and treated with 5 mol dm^{-3} hydrochloric acid (100 cm^3) for 10 min at room temperature. The dioxane was distilled off from the mixture and the aqueous solution extracted with ether (3 ×). The extract was washed with water and brine, dried and evaporated and the crude product was purified by silica gel chromatography eluting with 10% ethyl acetate–hexane. The *title compound* was obtained as a colourless oil (4.73 g, 88%) (Found: MH^+, 169.0868. $C_9H_{13}O_3$ requires *M*H, 169.0865); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 1735, 1665 and 1630; $\delta_H(CDCl_3)$ 1.54–1.87 (4 H, m, cyclohexene-H), 2.07–2.56 (4 H, m, cyclohexene-H), 3.90 (3 H, m, CO_2CH_3) and 7.08 (1 H, m, =CH).

Methyl 2-cyclohex-1-enyl-(*Z*)-2-methoxyiminoacetate 41a. The oxo ester **40a** (1 g) in ethanol (15 cm^3) and methoxylamine hydrochloride (1.19 g) was kept at room temperature for 12 h after which it was evaporated under reduced pressure and the residue partitioned between ethyl acetate and water. After separation and extraction of the organic phase with ethyl acetate, the combined extracts were washed with water and brine, dried and evaporated under reduced pressure. Silica gel chromatography of the residue afforded the *title compound* as a colourless oil (1.01 g, 86%) (Found: M^+, 197.1053. $C_{10}H_{15}NO_3$ requires *M*, 197.1052); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1725, 1625w, 1570w and 1425; $\delta_H(CDCl_3)$ 1.45–1.86 (4 H, m, cyclohexene-H), 1.96–2.48 (4 H, m, cyclohexene-H), 3.87 (3 H, s, CO_2CH_3), 3.92 (3 H, s, OCH_3) and 5.95 (1 H, m, =CH).

2-Cyclohex-1-enyl-(*Z*)-2-methoxyiminoacetic acid 42a. The ester **41a** (0.7 g) in methanol (15 cm^3) was hydrolysed according to the general procedure (E) to give the *title compound* as a colourless gum (0.6 g, 92%) (Found: M^+, 183.0901. $C_9H_{13}NO_3$ requires *M*, 183.0895); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3450w, 3300–2500br, 1750, 1715, 1625w and 1575w; $\delta_H(CDCl_3)$ 1.41–1.88 (4 H, m, cyclohexene-H), 1.97–2.49 (4 H, m, cyclohexene-H), 3.96 (3 H, s, OCH_3), 6.15 (1 H, m, =CH) and 11.61 (1 H, s, CO_2H).

Sodium 6β-[2-cyclohex-1-enyl-(*Z*)-2-methoxyiminoacetamido]penicillanate 43a. As outlined in general procedure (G)(ii), the acid **42a** (0.3 g) was coupled to 6-APA(TEA) and purified by HP20SS column chromatography to give the title compound as an amorphous white solid (0.51 g, 78%); ν_{max}(KBr)/cm^{-1} 1764, 1633, 1604, 1509, 1458w, 1401 and 1321; $\delta_H(D_2O)$ 1.49 (3 H, s, 2-CH_3), 1.60 (7 H, s and m, 2-CH_3 and cyclohexene-H), 2.17 (4 H, m, cyclohexene-H), 3.85 (3 H, s, OCH_3), 4.21 (1 H, s, 3-H), 5.57 (2 H, s, 5- and 6-H) and 6.18 (1 H, m, =CH); *m*/*z* (positive ion FAB, thioglycerol) 404 (MH^+) and 426 (MNa^+).

Methyl 2-(1-hydroxycyclopentyl)-2,2-dimethoxyacetate 39b. The procedure as outlined for example **39a** was repeated using cyclopentanone (5 g). Work-up and purification under the same conditions gave the title compound as a pale yellow oil (4.06 g, 52%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3560w, 1745, 1660w, 1640 and 1440; $\delta_H(CDCl_3)$ 1.40–1.97 (8 H, m, cyclopentyl-H), 2.56 (1 H, s, OH), 3.49 (6 H, s, OCH_3) and 3.85 (3 H, s, CO_2CH_3); *m*/*z* (CI) 236 (MNH_4^+).

Methyl 2-(cyclopent-1-enyl)-2-oxoacetate 40b. The hydroxy compound **39b** (4 g) was dehydrated and then hydrolysed to give the title compound as previously described for compound **40a**. After purification on silica gel with 5% ethyl acetate–hexane the product was obtained as a colourless oil (0.67 g, 24%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1725, 1665, 1600, 1420w and 1360w; $\delta_H(CDCl_3)$ 1.67–2.26 (2 H, m, cyclopentyl-H), 2.43–2.88 (4 H, m, cyclopentyl-H), 3.93 (3 H, s, CO_2CH_3) and 7.31 (1 H, m, =CH); *m*/*z* (CI) 309 ($2MH^+$).

Methyl 2-(cyclopent-1-enyl)-(*Z*)-2-methoxyiminoacetate 41b. The oxo ester **40b** (0.65 g) in methanol (10 cm^3) with methoxylamine hydrochloride (0.71 g) was converted into the *title compound* using the procedure outlined for compound **41a**. Following isolation and purification, the product was obtained as an oil (0.52 g, 67%) (Found: M^+, 183.0897. $C_9H_{13}NO_3$ requires *M*, 183.0895); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1730, 1610w, 1570w, 1435 and 1380; $\delta_H(CDCl_3)$ 1.75–2.26 (2 H, m, cyclopentene-H), 2.29–2.84 (4 H, m, cyclopentene-H), 3.94 (3 H, s, CO_2CH_3), 4.00 (3 H, s, OCH_3) and 6.12 (1 H, m, =CH).

2-Cyclopent-1-enyl-(*Z*)-2-methoxyiminoacetic acid 42b. The ester **41b** (0.51 g) was hydrolysed according to general procedure (E) in methanol (10 cm^3), to give the *title compound* as a pale yellow solid (0.47 g, 99%) (Found: M^+, 169.0736. $C_8H_{11}NO_3$ requires *M*, 169.0739); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3460w, 3350–2400br, 1760, 1725 and 1615w; $\delta_H(CDCl_3)$ 1.78–2.28 (2 H, m, cyclopentene-H), 2.35–2.89 (4 H, m, cyclopentene-H), 4.05 (3 H, s, OCH_3), 6.34 (1 H, m, =CH) and 11.40 (1 H, s, CO_2H).

Sodium 6β-[2-(cyclopent-1-enyl)-(*Z*)-2-methoxyiminoacetamido]penicillanate 43b. Using general procedure (G)(ii), the acid **43b** (0.43 g) was coupled to 6-APA(TEA). Following isolation and purification, the title compound was obtained as a white foam (0.54 g, 55%); ν_{max}(KBr)/cm^{-1} 1768, 1669, 1608, 1513, 1459w, 1401 and 1321; $\delta_H(D_2O)$ 1.49 (3 H, s, 2-CH_3), 1.59 (3 H, s, 2-CH_3), 1.87–1.99 (2 H, m, cyclopentene-H), 2.48 (4 H, m, cyclopentene-H), 3.86 (3 H, s, OCH_3), 4.21 (1 H, s, 3-H), 5.57 (2 H, s, 5- and 6-H) and 6.28 (1 H, br s, =CH); *m*/*z* (positive ion FAB, thioglycerol) 390 (MH^+) and 412 (MNa^+).

Methyl 2-(4-hydroxytetrahydropyran-4-yl)-2,2-dimethoxyacetate 39c. The procedure previously described **39a** was used with tetrahydropyran-4-one (3.36 g). In this case, however, after the mixture had been poured into water to quench the reaction, it was saturated with solid sodium chloride before being extracted with ethyl acetate (4 ×). The combined extracts were dried ($MgSO_4$) but not washed. After work-up the crude product was chromatographed on silica gel, eluting with 30% ethyl acetate–hexane to give the title compound as a colourless oil (6.09 g, 70%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3550w, 1740 and 1430; $\delta_H(CDCl_3)$ 1.15–2.03 (4 H, br m, tetrahydropyran-H), 2.94 (1 H, br s, OH), 3.51 (6 H, s, OCH_3), 3.74 (2 H, m, tetrahydropyran-H) and 3.85 (5 H, s and m, CO_2CH_3 and tetrahydropyran-H); *m*/*z* (CI) 252 (MNa^+).

Methyl 2-(2,3-dihydropyran-4-yl)-2-oxoacetate 40c. The hydroxy compound **39c** (6 g) was dehydrated as outlined for compound **40a** after which hydrolysis was achieved with dilute hydrochloric acid overnight at room temperature. Work-up involved saturating the reaction mixture with solid sodium chloride and extracting it with ethyl acetate. After purification, the title compound was isolated as a pale yellow liquid (1.91 g, 44%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1735, 1675, 1640, 1425 and 1380w; $\delta_H(CDCl_3)$ 2.25–2.61 (2 H, m, dihydropyran-3H), 3.91 (2 H, t, *J* 6.0, dihydropyran-2H), 3.97 (3 H, s, CO_2CH_3), 4.48 (2 H, dd, *J* 2.7, 5.3, dihydropyran-6H) and 7.29 (1 H, m, dihydropyran-5H); *m*/*z* (CI) 171 (MH^+).

Methyl 2-(2,3-dihydropyran-4-yl)-(*Z*)-2-methoxyiminoacetate 41c. As described for compound **41a**, the oxo compound **40c** (1 g) was converted into the title compound, a colourless oil (0.88 g, 75%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1735, 1430, 1380 and 1320; $\delta_H(CDCl_3)$ 2.27–2.51 (2 H, m, dihydropyran-3H), 3.80 (3 H, t, *J* 6.0, dihydropyran-2H), 3.84 (3 H, s, CO_2CH_3), 3.89 (3 H, s, OCH_3), 4.23 (2 H, dd, *J* 2.7, 5.0, dihydropyran-6H) and 5.85 (1 H, m, dihydropyran-5H); *m*/*z* (CI) 214 (MH^+).

2-(2,3-Dihydropyran-4-yl)-(*Z*)-2-methoxyiminoacetic acid 42c. The ester **41c** (0.78 g) in methanol was hydrolysed as outlined in general procedure (E). Work-up provided the *title compound* as a brown gum (0.74 g, 100%) (Found: M^+, 185.0683. $C_8H_{11}NO_4$ requires *M*, 185.0688); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3450br w, 3350–2500br, 1740sh and 1715; $\delta_H(CDCl_3)$

2.30–2.54 (2 H, m, dihydropyran-3H), 3.87 (2 H, t, *J* 6.0, dihydropyran-2H), 3.92 (3 H, s, CO_2CH_3), 4.31 (1 H, m, dihydropyran-6H) and 6.01 (1 H, m, dihydropyran-5H).

Sodium 6β-[2-(2,3-dihydropyran-4-yl)-(*Z*)-2-methoxyimino-acetamido]penicillanate 43c. The acid **42c** (0.4 g) was coupled to 6-APA(TEA) according to general procedure (G)(ii). After purification the title compound was obtained as a white foam (0.36 g, 41%); ν_{max}(KBr)/cm^{-1} 1768, 1663, 1602, 1459w, 1399 and 1319: $\delta_H(D_2O)$ 1.52 (3 H, s, 2-CH_3), 1.63 (3 H, s, 2-CH_3), 2.36 (2 H, m, dihydropyran-3H), 3.90 (2 H, t, *J* 5.6, dihydropyran-2H), 3.91 (3 H, s, OCH_3), 4.26 (1 H, s, 3-H), 4.32 (2 H, m, dihydropyran-6H), 5.61 (2 H, s, 5- and 6-H) and 6.19 (1 H, m, dihydropyran-5H); *m*/*z* (positive ion FAB, thioglycerol) 406 (MH^+) and 428 (MNa^+).

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